## MICROFLUIDIC DEVICE & METHODS FOR CONSTRUCTION & APPLICATION

### Field of Invention

The present invention relates to microfluidic devices and assemblies, methods for their construction with preferred embodiments and methodologies for the functional operation of
5  such.

### Background

The miniaturisation of chemical processes onto chip-based platforms enables a plethora of novel industrial
10  applications of existing and new chemistry, biochemistry, biomolecular science and particle science in both analysis, synthesis, assembly, decision making and computing. For example, molecular synthesis in micron-scale reactors benefits from (i) fast reaction kinetics, and (ii) high specific-areas
15  which facilitates greater control of and/or the use of highly exothermic reactions. Many advances in so-called, microfluidics have been made in recent years. Nevertheless, known devices and methods for the manipulation of fluids in miniaturised tubes, ducts and vessels have not met all the requirements of
20  industry. For example, sterilisation of, and the maintenance of an inert atmosphere in, microfluidic ducts remains a problem hampered by the materials from which many devices have been constructed. Additionally, the use of highly corrosive fluids and high temperatures again requires very strict attention to
25  the materials from which devices are fabricated, which, in turn, has serious implication for mass-manufacture of such devices and assemblies of such. Accordingly, special attention must be made to the suitability of constructional materials, the unit costs of manufacture, the rapidity of manufacture,
30  including tooling time for mass-production, and the translation of prototyping methods to mass-production. Constructional materials may include a glass, ceramics, stainless steel and other metals or alloys, silicon, polymers, paper and others. Glass-based substrates have been successfully manufactured, but

limited, to some extent, by the complexity of 3-dimensional geometry that is cost-effectively feasible. Also, external fluidic interconnect solutions remain crude. Photostructurable glasses (e.g. Fotoran made by Schoot) are very expensive,

5    sometimes 200x the cost of polymer substrates, and require several expensive and hazardous processing steps (e.g. use of HF) and specialised equipment (e.g. quartz based optics for lithography). Stainless steel chips can be manufactured but are limited with respect to 3-dimensional geometries attainable and

10   the surface quality possible, even with MicroElectroDischarge Machining, is frequently of insufficient resolution for microfluidic applications. The technique suffers from high unit-cost production and very limited availability of high resolution machining tools.   Silicon based microfluidic

15   devices, such as microreactors, have been made and benefit from available tools for silicon micromachining and fusion/anodic bonding procedures for bonding together multilayered devices. However, silicon is relatively expensive for mass fabrication of relatively large-format chips which may sometimes have a

20   short-lifespan. In addition, with exceptions, interconnect solutions remain inelegant and low-pressure and silicon denies the use of high field strength electric fields for the generation of electro-kinetic flow and certain molecular purification processes.

25       Many polymers (e.g. polysulphone, polycarbonate, polymethylmethacrylate) have been utilized for the fabrication of microreactors but most have been unsuitable for use with very aggressive liquids such as acids (e.g. nitric acid) and solvents (e.g. acetonitrile). In addition, the presence of

30   certain substances incorporated into the polymer matrix, such as plasticisers, may cause contamination during usage, as those compounds leach from the substrate matrix into the fluids within the ducts on the chip. Particularly, for many synthetic reactions the preferred substrate material would be a

35   fluoropolymer such as polytetrafluoroethylene $(C_2 F_4)_n$ [PTFE].

However PTFE and related variants are less easily micromachined to provide fluidic ducts of micron sized dimensions and very difficult to join with itself to form enclosed microreactor ducts. It is a purpose of the current invention disclosed

5  herein to provide a cost-effective resolution to the latter technical problems and enable the manufacture of suitable chip-based platforms for a wide range of industrial-scale diagnostic and synthesis operations.

Additionally, fluid flow in microscale ducts is

10  characterised by laminar flow conditions resulting from characteristically low Reynolds number regimes. This causes a problem with mixing of fluids and it is a purpose of the invention disclosed herein to provide a solution to that technically limiting issue. Also, fluid flow in micron scale

15  ducts is usually characterised by continuous streams of a given fluid phase. A contrasting method is where immiscible phase fluids are caused to flow along a duct in serial discontinuous aliquots. The generation of such segmented flow streams can be enabled by bringing together two streams of immiscible fluid

20  and causing them to merge at a so-called T-junction. This methodology has not met all the needs of industry. For example, such device configurations are frequently only stable for a narrow range of absolute flow rate conditions and relative flow rates of the immiscible phase liquids. In particular, it can

25  be difficult to control the generation of segmented flow streams with equal volumes of the immiscible phases, especially at low flow rates required by many applications. In addition, back-pressure can be considerable, especially in ducts of narrow (<100microns width, depth, both or diameter) and very

30  narrow (<25micron width, depth, both or diameter) dimensions. It is, therefore, a purpose of the invention disclosed herein, to provide improved solutions for the generation and subsequent manipulation of segmented flow streams in micron scale ducts.

Devices for the manipulation of fluids may be used for

35  analytical and synthesis purposes. Frequently, for a wide range

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of functional operations in both analytical and synthesis techniques it is necessary to elute precise volumes of fluids in a highly repeatable manner. For example, in titrations, 'split and mix' procedures, formation of microparticles such

5 as artificial cells and nanoparticles such as quantum dots. Because the volumes of liquids are usually very small it is frequently difficult to meet the exacting requirements of industry and solutions to date are generally insufficient to meet all needs. It is a further purpose of the invention

10 disclosed herein to provide device configurations and methods to improve substantially on those currently available. Furthermore, notwithstanding that devices and methods currently available for the controlled volumemetric elution of liquids, do not meet current needs, the subsequent manipulation of small

15 liquid volumes also requires improvements. In particular, there is a need to improve on techniques for altering the morphology of liquid samples, their conversion to non-liquid forms and the ability to encapsulate such small samples with films of other materials. It is also a further purpose of the invention

20 disclosed herein to provide further devices and associated methods to meet these needs.


## SUMMARY OF THE INVENTION

In accordance with a first aspect, a fluid manipulation device is described, which comprises a device composed of, at

25 least, two distinct fluoropolymer based substrate layers which may be composed of bulk fluoropolymer or fluoropolymer- or fluoropolymer-based coatings applied to other non-fluoropolymer bulk material substrate layers. The fluoropolymer bulk material layer(s) or applied coatings may include PTFE, Teflon®,

30 Teflon®AF, Teflon®NXT, Teflon® G, Teflon® PFA, Teflon® PFA HP Plus, Dyneon™, CYTOP®, Teflon® PFTE-silicone adhesive film (product 5490 of 3M). Additionally, in accordance with this first aspect, the fluid manipulation device is provided with at least two ducts, for the passage and/or storage of

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immiscible fluids.

It will be understood by those skilled in the art that either of the immiscible fluids may include aqueous matrices, supercritical fluids, supercritical Helium-3, organic solvents, ionic liquids, inert fluids such as perfluorinated alkanes, perfluoroethers (including but not limited to Galden®, Fluorinert™), oils, acids, gasses, suspensions of living (including cryo-preserved) cells, organelles and tissues, suspensions of preformed particulate materials such as chromatographic separations media, tissue scaffold precursors, etc.

It will further be understood by those skilled in the art that in turn the liquids may incorporate other components including (but not limited to): smaller particles of polymers (gelled or crosslinked) including non-biodegradable polymers such as (but not exclusively) ethylene vinyl acetate, poly(meth)acrylic acid, polyamides; biodegradable polymers including synthetic polymers such as polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters; natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof, albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof; bioadhesive polymers including bioerodible hydrogels, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates); antibodies, enzymes, abzymes, liposomes, antibiotics, nucleic acids and associated promoters, supercoiled DNA, oligonucleotides with <30 nucleotides, genes of more than 30 nucleotides with and without expression promoters; a wide range of pharmaceutical molecules, living cells, organelles, bacteria, viruses, viral vectors, metallic particles, magnetic particles, quantum dots, radioactive particles or radioactive-tagged molecules, fluorescent- chemi-luminescent- and/or bio-luminescent

particles, molecularly imprinted polymers, tissue scaffold precursors, semiconductor devices, micron-scale RF transponders, liquid crystals, porous silica particles such as beads, porous silicon particles and others.

5          The two ducts are arranged to be separated by a thin partition (typically less than 500um, preferably less than 10um and most preferably less than 1 micron) until a point at which the partition is no longer present. The two parallel, closely associated ducts join to a third duct (hereafter called the

10   commmon duct) measuring, optionally 500microns to 100 nanometeres in either cross-sectional dimensions of width, depth, mean diameter, but of a length, at least, ten times the minimum width of either the two preceding ducts, so that the different fluids moving in parallel flow formation along the

15   third duct attain a very stable flow profile free from shear forces and microturbulence at their interfacial juncture. The cross-sectional area of the third duct is preferably, equal to the sum of the cross-sectional area of the two preceding ducts. The third duct axially joins a fourth duct (hereafter called

20   the segmented flow duct) that is of cross-sectional area and width at least 25% most preferably 50%, that of the third duct. At the juncture of the said common duct and constriction duct, the permanent or temporary, 2- or 3- dimensional, constrictions in cross-sectional geometry are configured such that when two

25   or more streams of immiscible fluids are caused to move within the common duct the fluids may be forced from a laminar unmixed flow condition to form contiguous serial segments of the immmiscible liquids in the fourth duct. The immiscible liquids may be caused to move within the ducts by means of applied

30   pressure- (negative or positive), electrokinetic forces (where charged functional groups are provided on the walls of the fluid bearing ducts), displacement (e.g. by the action of a plunger caused to move by the application of a mechanical force from a human being) or centrifugal forces (e.g. by means of

35   rotation about an axis). The absolute rate of flow of fluid

segments may be adjusted by varying, in equal amounts, the flow
rates of the immiscible fluids. The relative volumes of the
immiscible liquids formed as fluid segments may be adjusted by
varying the relative flow rates of the same immiscible liquids.
5  At the point at which the two or more ducts merge the
immiscible fluids remain substantially in parallel (laminar)
formation and continue in such formation as they are caused to
move along the common duct. When or where the common duct(s)
is/are physically constricted (permanent or temporarily) the
10  liquid components are forced together in a more constrained
environment. At the point(s) of constriction the laminar flow
may become interrupted such that one liquid will move into the
narrower section of the duct first to be followed by aliquots
of the second and other immiscible liquids in a manner
15  determined by the relative contact angles of the fluid
component phases, the surface energy of the fluoropolymer
material, the force applied to propel the fluids, elastic
properties of the component liquid phases and the precise 2-
and 3- dimensional geometrical nature of the physical
20  constriction. This allows the selective passage of given phase
liquids to pass along the duct establishing a repeated and
ordered segmented stream of liquid phase materials. Because the
flow is manipulated in a controlled volume environment and
because all the environmental parameters (e.g. but not limited
25  to pressure, flow-rate, temperature, humidity, surface energy)
can be finely controlled, fluid segments are produced to a
highly accurate volume and morphological specification. This
fine control of fluid segment volume and geometrical morphology
gives rise to many advantages that can be of benefit to
30  analytical and synthesis operations when conducted in the fluid
based device. The absolute lengths of fluid segments may be
controlled in a time-varying dynamic manner by controlling the
absolute flow rate of the fluids into and along the common
fluid duct. The relative lengths of the fluid segments may be
35  determined by providing ducts of differing cross-sectional area

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for the separated flow streams prior to merger in the common
fluid duct. The relative lengths of the fluid segments may be
determined in a time-varying dynamic manner by controlling the
relative flow rates of the separated flow streams prior to
5  merger in the common fluid duct.

Additionally, in accordance with this first aspect, the
said constriction feature is utilised, at least (but not
exclusively), for the controlled volume elution of immiscible
fluid phase components. Additionally, in accordance with this
10 first aspect, the device is designed and manufactured,
specifically and uniquely, for the creation of, and functional
operation with, at least one segmented flow stream of
immiscible fluid-based matrices, which are favourably enabled
by the use of low surface energy fluoropolymer-based
15 manufacturing materials. Additionally, in accordance with this
first aspect, the said fluidic ducts are machined, most
preferably by means of reactive ion etching, even more
preferably with the assistance of an inductively coupled
plasma, even more preferably where the fluoropolymer-based or
20 coated substrates and/or reactive  plasma are cooled with a
cryo- facility. A particular advantage of utilising reactive
ion etching of PTFE and related polymers is that it is
frequently difficult to micromachine by other means.

For instance, because pure PTFE it is not a
25 thermoplastic it is not easily moldable and as such does not
lend itself to microstructuring by embossing or  injection
molding techniques. When ablated by laser machining [e.g.
copper vapour, excimer] the ablated surface is often highly
irregular and generally unsuited to microfluidics chemistry and
30 other applications. Furthermore, reactive ion etching is a
parallel process in that all areas of substrate exposed to the
plasma are etched at the same time which renders it suitable to
manufacturing of substrates suitable for microfluidics,
microchemistry and other applications where a relatively large
35 substrate format is required. This contrasts with excimer laser

micromachining which is frequently used (though not always) in a serial manner where the laser beam is moved over a workpiece to define a geometry.

Additionally, the reactive ion etching process lends itself to mass-production by virtue of available equipment commonly employed for silicon and silica micromachining. Automated cassette-loading systems with large wafers could facilitate high-volume production with the advantage that complex, high aspect ratio, nanoscale features could be incorporated which is not easily achieved with medium volume micromanufacturing techniques such as injection micromolding. Reactive ion etching of fluoropolymers may be used as both a prototyping and mass-manufacturing process. This provides for a seamless transfer of processing technology to different scales of production. This holds an advantage over embossing and injection molding where separate tools have first to be constructed which is both time-consuming and expensive. Because manufacturing costs of components made by etching are determined, in part, by processing time, accelerated etching times allows faster processing operation to be done with the result of higher unit throughput. Unit costs of manufacture are thus reduced. Less preferably, the ducts may be machined by CNC milling, preferably where substrates are cooled to lessen the surface roughness of the finished surfaces. Less preferably the said ducts may be machined in thermoplastic variants of fluoropolymer based substrates by means of hot embossing, injection molding, laser ablation (excimer or copper vapour) and broad area ion-beam milling.

Additionally, in accordance with this first aspect, the immiscible fluids are located and manipulated within the said ducts, the device having no operative fluid ports for communication external to the device. Additionally, in accordance with this first aspect, the device incorporates a passive (batteryless and wireless) radio frequency transponder device to provide individual device identification.

Additionally, in accordance with this first aspect, the fluoropolymer-based or fluoropolymer-coated layers are encased under compression within a recess machined in between two or more encasement layers of a dissimilar thermoplastic (e.g.
5 PMMA, polycarbonate) that may be mechanically bonded together or joined by thermal, thermo-compression or thermally assisted (e.g. by inductive heating) compression bonding. The fluoropolymer and /or fluoropolymer based layers are provided with a (combined) slightly larger (by 10 microns and up to 100
10 microns) thickness dimension than the depth of a recess provided in the separate casement. During thermal bonding of the casement the fluoropolymer-based insert becomes embedded within it such that upon cooling the fluoropolymer-based layers are sandwiched at high pressure. This results in a mating
15 between the fluoropolymer-based surfaces that are fluid tight at levels highly useable for low-pressure applications, particularly in chemistry and biotechnology. Preferred embodiments of the device may be utilised for a wide range of industrial research, development and manufacturing operations,
20 as a singular device, or as 2 or more operating in serial or parallel arrangements and/or as a device interfaced with other equipment. Additionally, in accordance with this first aspect the device is manufactured substantially in a planar layer-like format, each of which are of a geometrical area >350square
25 millimetres, typically 8000 square millimetres and 32000 square millimetres, most preferably in circular format, less preferably in rectangular format, each layer being at least 40 microns in thickness, with one layer being at least 475 microns in thickness.
30      In accordance with another aspect, the fluid manipulation device is configured with a port suitable for fluidic communication with the external environment and/or equipment, and /or mammalian body. Such equipment may include, but not be limited to, rotary, piston and other displacement
35 pumps; vacuum lines, gravitationally driven fluid streams;

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analytical apparatus for mass spectrometry, liquid chromatography, particle characterisation, nuclear magnetic resonance, impedance spectroscopy, UV/VIS/IR absorption; and sample storage (including but not limited to microtitre
5  plates, vials, collection vessels, porous materials in bead, strip, card and disc-like formats, dry storage plates including porous silicon microspot arrays).

In accordance with another aspect, the fluid manipulation device may be configured with two ports suitable
10  for said fluidic connection to external environment, mammalian body and equipment.

In accordance with another aspect, the fluid manipulation device may be configured with three ports suitable for said fluidic connection to external environment, mammalian
15  body and equipment.

In accordance with another aspect, the fluid manipulation device may be configured with more than three ports suitable for said fluidic connection to external environment, mammalian body and equipment.
20        In accordance with another aspect, the fluid manipulation device may be manufactured, such that one or more substrate layers, formed of bulk fluoropolymer or fluoropolymer coated material, utilises a thermoplastic fluoropolymer that melts when raised above its glass transition temperature. The
25  juxtaposed layers may be hermetically joined by the inductive heating (upon exposure to strong electromagnetic fields) of a metallic (e.g., but not limited to, titanium, aluminium, chromium) interlayer film deposited on to one or more of the said layers, and which, upon inductive heating to above the
30  glass transition temperature, causes local melting of the thermoplastic fluoropolymer materials, which upon subsequent cooling (after removal of the inductive heating element), forms a hermetic and mechanically secure seal.

In accordance with another aspect, the fluid
35  manipulation device may be manufactured, such that one or more

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substrate layers, formed of bulk fluoropolymer or fluoropolymer coated material, utilises a thermoplastic variant of fluoropolymer that melts when raised above its glass transition temperature. The juxtaposed layers may be hermetically joined

5  by the combined application of heat and pressure, raising the surface temperatures of the layers where they adjoin to at least 5°C above the glass transition temperature. This causes local melting of the thermoplastic fluoropolymer materials, which, upon subsequent cooling, forms a hermetic and

10 mechanically secure seal.

    In accordance with another aspect, the fluid manipulation device may be provided with the additional incorporation of protrusions of the thermoplastic encasement material, that, when assembled, fit through via holes provided

15 in the fluoropolymer-based or -coated substrates, and mate with corresponding protrusions from the opposite side. This mating may be caused by simple butt joints or by a number of geometrical arrangements where 'plug and socket' type modifications to the protrusions are made. These protrusions

20 may be securely but non-permanently joined, simply by mechanical means, and/or made permanent by thermo-compression bonding. The joined protrusions act to provide additional strength to the hybrid assembly particularly on large format structures and prevent fluid escape between the layered

25 fluoropolymer-based or -coated parts. Small through-vias may be provided through 'plug and socket' type protrusions such that an oversized pin (preferably made, though not exclusively, from stainless steel) driven through the vias will enhance physical contact and the security of the fastening achieved, or by use

30 of a bolt and nut tightened to secure a mechanical and hermetic fastening.

    In accordance with another aspect, the fluid manipulation device may be manufactured where one or all of the fluoropolymer based layers comprises a fluoropolymer-based thin

35 (typically 1-3microns) film (e.g. Teflon® AF) film applied to

an optically transmissive material (e.g. but not limited to borosilicate glass, Pyrex, QC PMMA) such that the fluid contained within the fluid ducts and receptacles is enclosed completely by fluoropolymer based materials whilst allowing for
5   the passage of light which may be used for imaging, and/or photo-excitation of devices (e.g. actuators) or phenomena (e.g. photocatalysis, gelling). This thin film may be applied by means of various techniques such as spin-coating [solutions or nanoparticle suspensions], RF magnetron sputtering or Plasma
10  Enhanced Chemical Vapour Deposition, optionally followed by an annealing process, also optionally followed by exposure to an oxygen or oxygen/ammonia plasma to enhance adhesive and/ or (chemical) functionalisation qualities.

In accordance with another aspect, the fluid
15  manipulation device may be provided with fluidic ducts and receptacles that are machined in both sides of the fluoropolymer based or coated substrate layers providing for double sided fluidic substrates. In such devices a third fluoropolymer-based or -coated layer is required to provide a
20  fluidic environment where all parts of the fluidic system are provided by fluoropolymer-based materials.

In accordance with another aspect, the fluid manipulation device may be provided with one or more fluidic vias through the substrate layers providing fluidic
25  .communication between both sides of the substrate.

In accordance with another aspect, the fluid manipulation device may be manufactured with two or more single- or double-sided fluidic substrate layers that may be stacked upon each other, directly or indirectly (optionally,
30  with a separating layer) to create a multi-layer assemblage (e.g. up to 1000 or more substrate layers). These layers may have identical or dissimilar function. Such devices may incorporate one or more additional substrate layers, the function of which is to act as a manifold for distributing
35  fluid based matrices from one or more input and output tubes to

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one or many ducts configured to generate segmented flow streams.

In accordance with another aspect, the fluid manipulation device may be manufactured such that, additional, 5 smaller modules may be attached aboard the device so as to provide additional functionality. These modules may incorporate sensors (e.g. impedance sensor measuring liquid purity), actuators (e.g. diverters of fluid flow) reservoirs of reagents (e.g. fluids, particles, etc.) or may function to provide 10 clusters of one or more additional input-output fluid ports to the fluidic substrate layers or stack of substrate layers. These modules may be attached by means of one or more plug-like projections from the smaller modules which respectively mate with socket-like receptacles provided in the substrate layers, 15 or encasement layers of the device. The modules may be stacked one upon another as described in International Patent application WO02/060810. In a preferred embodiment, 6 plug-like projections are arranged peripherally and circumferentially on the underside of disc-like modules at separation angles of 60°, 20 but the number, position and format may take many variant forms. The plug-socket connectors allow for mechanical fastening, fluid transport, electrical connectivity, optical connectivity, the intimate application of electrical and magnetic fields and the intimate application of inductive 25 heating.

In accordance with another aspect, the fluid manipulation device may be manufactured where the encasement layers are manufactured from a non-polymer material (most preferably, though not exclusively stainless steel) joined not 30 by thermocompression bonding but rather through the use of one or more bolts extending between casement layers so as to form a mechanically tight assemblage of fluid substrate layers.

In accordance with another aspect, the fluid manipulation device may be manufactured where no encasement layer is 35 provided, the fluid substrate layers being preferably

constructed from thermoplastic fluoropolymer (e.g. Teflon® AF, Teflon® NXT, Teflon® G, Teflon® HP, Teflon® HP Plus) bulk materials or thermoplastic fluoropolymer coated material of another polymer (e.g., but not exclusively, polycarbonate,

5 polymethylmethacrylate), the said layers being joined by thermal, thermally assisted, ultrasonically assisted or thermocompression bonding.

In accordance with another aspect, the fluid manipulation device may be provided with a fluid port such that

10 one of the fluid phase components is introduced on to the device from an external supply.

In accordance with another aspect, the fluid manipulation device may be provided with two fluid ports such that two of the fluid phase components are introduced on to the

15 device from external supplies.

In accordance with another aspect, the fluid manipulation device may be provided with sufficient ports that all of the fluid phase components are introduced on to the device from external supplies.

20      In accordance with another aspect, the fluid manipulation device may be provided with a fluid port such that one of the fluid phase components, or products of, may be exported  from the device to an external location (such as a collection vessel, instrument, mammalian body, atmosphere, or

25 vacuum).

In accordance with another aspect, the fluid manipulation device may be provided with two fluid ports such that  two of the fluid phase components, or products of, may be exported  from the device to an external location (such as a

30 collection vessel, instrument, mammalian body, atmosphere, vacuum).

In accordance with another aspect, the fluid manipulation device may be provided with many fluid ports such that  three or more of the fluid phase components, or products

35 of, may be exported  from the device to an external location

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(such as a collection vessel, instrument, mammalian body, atmosphere, vacuum).

In accordance with another aspect, the fluid manipulation device may be manufactured such that one or more
5  geometrical constrictions in a fluid duct, designed to cause segmented flow of immiscible fluids, are replaced or enhanced with a permanent step change in surface energy achieved by the differential patterning of the surface energy states. These altered energy states may be achieved in a time-non-varying
10  manner by spatially-variable exposure of the surfaces to reactive plasmas, the grafting of functional moieties or the selective patterning of a dissimilar fluoropolymer film over the substrate layer. One example is where a thin film of Teflon® AF (a copolymer of Teflon® and
15  perfluoro-2,2-dimethyl-1,3-dioxole) is used to coat the surface of the fluidic ducts but where an electronegative charge is selectively implanted, in a spatially-variable manner, in the film by, for example, use of a back-lighted thyratron electron gun.
20        In accordance with another aspect, the fluid manipulation device may be manufactured such that one or more geometrical constrictions in a fluid duct, designed to cause segmented flow of immiscible fluids, is replaced with a time-varying step change in surface energy that may be actuated
25  to cause otherwise laminar flows of immiscible phase liquids to reconfigure to segmented flow. This may be achieved by electro-wetting where one or more insulated electrodes apply and determines the shape of an electric field at given points within the fluidic duct(s) so as to cause, otherwise, laminar
30  flows of immiscible phase liquids to reconfigure to segmented flow. A similar result may also be achieved by photo-wetting where one or more addressable photo-responsive surfaces are incorporated within the common fluidic duct such that when excited by illumination with incident light, the altered
35  surface energy states, causes otherwise laminar flows of

immiscible phase liquids to reconfigure to segmented flow. These altered energy states may be induced in, for example, spatially-variably deposited thin films of silicon (e.g. by electron beam deposition) or photo-responsive functional
5   moieties grafted on to the surface of the fluoropolymer or fluoropolymer coated ducts.

In accordance with another aspect, the fluid manipulation device may be manufactured where the geometrical constriction in a fluid duct, designed to cause segmented flow
10  of immiscible fluids, is enhanced or replaced with a cruciform junction of fluidic ducts whereby a separate duct crosses the path of the common fluidic duct. An intermittent DC voltage applied to the separate fluid duct which crosses the common fluid duct will cause the momentary, preferential,
15  electro-kinetic movement of aqueous fluids from the common fluidic duct, resulting in the conversion of otherwise laminar flows of immiscible phase liquids to reconfigure to segmented flow within the common fluidic duct.

In accordance with another aspect, the fluid
20  manipulation device may be provided with one or more ducts, in which segmented flow has been created, which said ducts split with partitions at a given point, to provide two or more separate ducts (referred to here for convenience as 'shredding ducts') which are smaller in cross-sectional dimensions. This
25  partitioning into 'shredding' ducts cause fluid segments to break into smaller segments thus having a 'shredding effect'. One or more of the smaller ducts may also be similarly partitioned again, so causing further division of the fluid segments. The 2- and 3-dimensional geometries of the 'shredding
30  ducts' may be selected such that the relative morphology of the fluid segments remains constant, or such that the morphology is significantly altered (for example to produce filamentous fluid segments). The smaller ducts may reduce in size to as little as 70 nanometres in width and 200nm in depth.
35              In accordance with another aspect, the fluid

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manipulation device may be provided with two or more ducts, each said duct carrying immiscible component fluids, each of which have been configured to flow in segmented formation (hereafter called feeder ducts). The segmented flow ducts merge

5  to form a common duct resulting in the coalescence of individual fluid segments, of the same phase, from the two or more previously separated segmented flow (feeder) streams. Each coalesced fluid segment assumes the internal circulation pattern typical of segmented flow, thus causing rapid mixing

10  within a given segment. The device may be used to react/ mix or dilute fluid phase components derived from the feeder ducts. The device may be used to advantageously (rapidly) mix aqueous solutions/suspensions which, otherwise, would remain relatively unmixed for a long duration whilst constrained under the

15  laminar flow conditions that usually dominate in microfluidic environments. The device may be advantageously utilized to dilute one given fluid phase fed from one of the feeder ducts by altering the volume ratio of the immiscible phase components in the other feeder ducts.

20        In accordance with another aspect, the fluid manipulation device may be provided where the unit of operation for mixing dilution is replicated at least two or more times on the same device substrate or stack of substrates. Particularly, the multisite mixing and/or dilution capability allows the

25  device to simultaneously mix and/or dilute reagents prior to dispensing into or onto a number of integral or external multiwell or multisite devices for the purposes of further processing operations (e.g. into the wells of a microtitre plate) or storage (e.g. on to selective sites of a MALDI plate

30  device, or a porous material such as a bead, ceramic plate or layer of porous silicon).

In accordance with another aspect, the fluid manipulation device may be configured such that two or more aqueous flow streams are caused to flow along a common duct,

35  which merges (as a constriction- or T-junction) with one or

more separate ducts carrying an immiscible fluid, resulting in the formation of a segmented flow stream, the internal vortex flow patterns of which fluid segments causes the rapid mixing of the 2 or more aqueous liquid matrices, which would, under

5  laminar flow conditions, otherwise, remain unmixed for a considerable period depending upon the Reynolds Number.

In accordance with another aspect, the fluid manipulation device may be manufactured such that a first duct carrying a segmented flow stream is joined at a juncture, by

10  one or more additional ducts, preferably in serial succession, at which points of juncture the first duct is, optionally, geometrically enlarged in cross-sectional area (either in width, depth or both), and where, during the passage of fluid flow along the first duct, supplementary aliquots of fluid

15  from the additional ducts may be injected or added to the volume of selected, or all, fluid segments in the first duct. The supplementary fluid added to the fluid segments is caused to mix rapidly by virtue of the internal flow vortex within the component fluid segments. The flow of fluid from the

20  additional duct into the first duct may be caused by passive means where like solutions momentarily contact and 'pull off' an additional aliquot of fluid from the additional duct. Equally, the flow of fluid from the additional ducts may be caused by propulsive methods including positive displacement

25  pump, electrokinetic injection (e.g. actuated by monitoring the passage of individual fluid segments in the first duct), piezoelectric actuator, and others.

In accordance with another aspect, the fluid manipulation device may be manufactured with one or more

30  pressure sensors in the form of capillary gas-filled manometers. These are constructed as blind tributary ducts arranged at any angle between 0° and 180° to the first microfluidic duct along which fluids are caused to move. The first microfluidic duct may be any duct formed on the device

35  for manipulation of fluids where there is non-segmented flow.

The tributary ducts are preferably filled with a relatively inert gas such as, though not exclusively, argon, helium or nitrogen. Fluid within the first fluidic duct will rise into the tributary duct according to the prevailing pressure
5   conditions. The tributary ducts function according to the principle that a change in gas volume provides a change in fluid pressure, i.e., an increase in fluidic pressure gives a decrease in gas volume. Therefore, by knowing the cross sectional area and length of the blind tributary ducts, the
10  initial volume can be calculated and with the displacement of the fluid in the channel the increase/ decrease of the pressure affected volume can be calculated. With liquid in the first microfluidic duct, the liquid level within the tributary duct rises to an initial pressure setting. According to pressure
15  changes (for example, during fluid movement caused by the application of pressure) the pressure of the fluid increases and the fluid moves further along the tributary duct changing the volume of the trapped gas. Other geometrical arrangements of more than one tributary duct can provide information on
20  fluid flow rate by indicating  the pressure drop over predetermined length or over a fluid resistor caused by a constriction in the first fluid duct. The capillary manometers may be provide in a linear, serpentined, curved or coiled format and meniscus movements visualised by an optical device
25  such as a CCD.

       In accordance with another aspect, the fluid manipulation device may be provided with one or more peltier based microcooler devices which cause parts of the fluid duct(s) and their contents to be reduced in temperature. This
30  may be particularly useful for a range of (bio)physiochemical operations such as replication of nucleotide sequences, protein crystallisation, polymer cross-linking by light- and ion-exchange means, exothermic chemical reactions and others.

       In accordance with another aspect, the fluid
35  manipulation device may be manufactured where a first duct

carrying a segmented flow stream is constricted and reduced in cross-sectional area (width, depth, both, or diameter) so as to cause fluid segments formed in the first duct before the constriction, to change shape, such as becoming elongated or

5 altered in cross-sectional profile (for instance, assuming a stellar-like or cruciform section). Optionally, such fluid segments may be caused to become less fluid in nature by partial or total polymerisation and/or gelling, by the selective exposure to light (most preferably ultraviolet, less

10 preferably visible and infrared light) temperature alteration, ionic composition of external environment, or the presence of gelling/polymerisation or encapsulation agents injected into the flow stream or directly into individual fluid segments.

In accordance with another aspect, the fluid

15 manipulation device may be manufactured where a first duct carrying a segmented flow stream is expanded in geometrical cross-sectional area (either in width, depth, both or mean diameter) by an amount sufficient to cause fluid segments in the first duct, before the point of expansion, to change shape

20 from non-spherical forms, to a spherical format, the boundary layers of each segment no longer assuming intimate shear contact with the duct walls. Configured in this manner, the rapid internal vortex flow that can be induced within the fluid packets of segmented flow (due to shear contact with the

25 duct walls during passage along the said duct) is lost as fluid segments assume a spherical format with no intimate shear contact with the duct walls. Following this methodology contiguous fluid segments become converted to trains of spheres of highly similar volume. The trains of spheres may be

30 collected for usage or further processed.

It will be understood by those skilled in the art, given the benefit of this disclosure, that an ability to produce droplets of highly reproducible volume and shape has innumerable applications and uses where size and shape affect

35 their function. An example is the size of particles (e.g.

liposomes, alginate beads) designed to deliver pharmaceuticals and genes to target tissues within the mammalian body. Similarly the production of droplets of highly reproducible volume and shape is particularly useful in the production of
5  foodstuffs, cosmetics, detergents and skin-care products.

In accordance with another aspect, the fluid manipulation device may be used where the contents (or part) of liquid segments are polymerised or crosslinked by exposure to electromagnetic radiation, [in the wavelength range 200-700 nm,
10 most preferably in the long wavelength ultraviolet (LWUV) range or visible range, 320 nm or higher, and most preferably between about 365 and 514 nm]. Dyes and co-catalysts, such as amines, for initiating polymerisation may also be included in the liquid segments such that upon exposure to visible or long-wave
15 ultraviolet light, active species can be generated. Light absorption by the dye causes the dye to assume a triplet state, which subsequently reacts with the amine to form an active species which initiates polymerization.

In accordance with another aspect, the fluid
20 manipulation device may be used where the contents (or part) of liquid segments are ionically cross-linked by the exposure to appropriate gelling agents delivered through a subsidiary duct, joining the main duct carrying the segmented flow stream(s), at, or just after, the point of duct expansion. One example to
25 illustrate this is the gelling of (typically, 1-12%) alginate solutions [which may in turn incorporate smaller particles or other components as described herein], where a segmented flow stream of sodium alginate and an immiscible phase such as an oil or perfluoroethers such as Galdens®, Fluoinert™, or organic
30 solvent such as methylene chloride is created in a duct which incorporates an expansion zone. At the expansion zone the segments of alginate emerge as droplets (preferably, though not exclusively as spherical droplets) suspended within the flow of the immiscible phase, and due to the added exposure to weak
35 calcium levels (typically 10mM -200mM Ca $Cl_2$) delivered in to

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the expansion duct from the subsidiary duct, the alginate droplets gel (optionally entrapping or immobilizing, for instance, cells or genes suspended within the alginate matrix) as calcium ions preferentially replace the sodium ions in the
5   alginate. This example method produces gelled (non-exclusively) sphere-like droplets of highly precise and repeatable dimensions, not easily attainable by other means and lends considerable advantage to the use of alginate which is widely used in food, pharmaceutical, textile, and paper products,
10  exploiting the properties for thickening, stabilizing, gel-forming, and film-forming. For instance, in gene delivery systems it is desirable to construct micron and nanometre sized capsules with high precision of morphological dimensions, thus assisting their targeted delivery to specific tissues within
15  the mammalian body. As a further specific illustration, microparticles formed in this way, which are advantageously suitable for gene delivery, may be comprised of biocompatible bio-erodible hydrogels including polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate,
20  chitosan, in which are suspended genes of greater than 30 nucleotides with specific expression promoters.

In accordance with another aspect, the fluid manipulation device may be used to function for the elution of liquid solutions and suspensions into gelled, polymerised,
25  solidified materials in spherical volumes ranging from 0.1 cubic millimetre to 0.003cubic micrometers of repeatably high precision volume.

In accordance with another aspect, the fluid manipulation device may be configured such that a first duct,
30  along which is caused to move a segmented flow stream of immiscible fluids, is temporarily merged with another duct carrying one liquid phase component similar in phase to one of the liquid phase components located within the first duct. After the point of merger the flow streams remain in a common
35  duct before then exiting through two separate ducts formed by

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splitting the common duct in two. At the point where the two ducts merge, the flow streams meet but remain substantially separated due to the laminar flow resulting from the low Reynolds number conditions. Despite this laminar flow, there is
5  an attraction between like fluids in the two streams that merge at the junction, causing like fluids to coalesce and move out through one exit duct and the unlike fluids to move out  via the other exit duct.

   In accordance with another aspect, the fluid
10 manipulation device may be used for the creation of quantum dots, and assemblages thereof, from a liquid phase.  To illustrate this process, an illustrative example is described. In this embodiment, an organometallic precursor triocytlphosphine oxide (TOPO) are provided in separate
15 immiscible fluids, formed on the device into segmented flow streams.  The fluid segments containing the triocytlphosphine oxide (TOPO) may be selectively heated by incorporating an energy absorbing dye (e.g. an infrared-absorbing dye) or particulate material (titanium, platinum, palladium, to absorb
20 light or induced electric fields) within the same fluid segments or the entire train of fluid segments may be heated by the direct application of a heating element (e.g. electrical resistance heater). Contiguous fluid segments in the segmented flow of the device enjoy a physical contact at the end of each
25 fluid segment. As the segments are caused to move along the reactor this interfacial boundary is highly dynamic, and continually renewing. Mass transfer across this interfacial boundary can therefore be very rapid and is dependent upon several factors including the speed at which the segments are
30 caused to move in the device. The rate at which the organic precursor is transferred to adjacent fluid segments containing the triocytlphosphine oxide (TOPO) may be controlled by adjusting the flow rate of the segmented flow stream. Equally, the mass transfer may be controlled by altering the geometry of
35 the duct carrying the segmented flow such that an expansion

(which may be for only a selected part of the duct) in the size
of the duct will cause elongate fluid segments to assume a
spherical form as freely moving droplets that have no contact
with the duct walls, and thereby do not enjoy the rapid
5   internal vortex flow. Accordingly, mass-transfer is adjusted.

It will be apparent to those skilled in the art, given
the benefit of this disclosure, that duct geometries may be
designed to provide precision control of mass-transfer and by
which means assume accurate control of quantum dot nucleation
10  conditions. Quantum dots are nano-scale crystalline structures
which can transform the colour of light and have numerous
applications ranging from computing, photonic and materials
coating to biotechnology. The energy states of the dots can be
largely controlled by the size of the dot.  Typically, this is
15  achieved by introducing a desired organometallic precursor into
heated triocytlphosphine oxide (TOPO) that has been vigorously
stirred under an inert atmosphere. As nucleation proceeds to
further shell growth the solution changes through different
colour regimes as the dot size increases. Precision control of
20  quantum dot formation has been difficult to refine because it
is not readily possible to achieve 'snap' control of nucleation
thermal and other conditions. Subsequently, quantum dots are
usually sized post-synthesis to obtain particles of specific
character. Quantum dot formation in the device described offers
25  a step-change advantage where thermal control may be exercised
with considerable precision. In particular, with segmented flow
the mass transfer can be almost instantaneously directed by
switching between vortex flow and laminar flow, the latter in
an expansion zone of the duct(s). In addition the fluoropolymer
30  construction of the device allows the reaction to be conducted
at sufficiently high temperatures, which would not be possible
were it constructed from other polymers (e.g. polycarbonate),
and the enclosed nature of the ducts can be utilised to control
the required inert atmosphere conditions. The processing can be
35  further elaborated by the introduction of further reagents

through tributary ducts to the main duct carrying the segmented flow stream, for instance to alter the composition of subsequent layers around the core of the quantum dot.


## BRIEF DESCRIPTION OF THE DRAWINGS

5          Embodiments of the present invention will now be described by way of examples only and with reference to the accompanying drawings, in which:

FIGS 1A-1E show several variants of a first embodiment of fluid manipulation device in accordance with the invention;

10         FIGS 2A-2B are sectional and plan views of a second embodiment of fluid manipulation device;

FIGS 3A-3B are plan and sectional views of a third embodiment of fluid manipulation device;

FIGS 4A-4B are plan and sectional views of a fourth

15 embodiment of fluid manipulation device;

FIGS 5A-5D are views of a fifth embodiment of fluid manipulation device;

FIG 6 is a side view of a daughterboard for fluid manipulation devices of the present invention;

20         FIG 7 is a plan view of a sixth embodiment of fluid manipulation device;

FIGS 8A-8C show a seventh embodiment of fluid manipulation device;

FIGS 9A-9C show variants of the device of Figs 8A-8C;

25         FIGS 10A-10B show an eighth embodiment of fluid manipulation device;

FIGS 11A-11B show variants of a ninth embodiment of fluid manipulation device;

FIG 12 shows a tenth embodiment of fluid manipulation

30 device;

FIG 13 shows an eleventh embodiment of fluid manipulation device;

FIG 14 shows a twelfth embodiment of fluid manipulation device;

27

FIGS 15A-15C show a thirteenth embodiment of fluid manipulation device;

FIGS 16A-16B show a fourteenth embodiment of fluid manipulation device;

5      FIG 17 shows a fifteenth embodiment of fluid manipulation device;

FIG 18 shows a sixteenth embodiment of fluid manipulation device;

FIG 19 shows a seventeenth embodiment of fluid
10 manipulation device; and

FIGS 20A - 20D show variants of an eighteenth embodiment of fluid manipulation device.


## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

15      Figs 1A-1E show various configurations for a fluid manipulation device. In FIGS 1A-1B a first fluoropolymer-based or -coated substrate 1 and a second fluoropolymer-based or -coated substrate 2 are held in intimate juxtaposition by various means. In FIGS 1A-1E the substrates are encased within
20 two or more additional layers of casement material 3. The substrate layers sit within a cavity machined within the casement layers, the depth of which is slightly shorter than the combined thickness of the substrate layers. The casement material may extend, as projections 4, through vias in the
25 substrate layers joining compatible protrusions on another corresponding casement layer. The casement material is a thermoplastic polymer welded together by thermocompression bonding or by inductive heating, of an optional thin metallic film 5 that may be applied to one of the two layers, to
30 >Tg+5°C of the thermoplastic polymer. At the points where the casement materials meet, the surface may be provided with interlocking inter-digitations useful for alignment, allowing for variations in the thickness of substrate layers and enhanced surface area for joining. Alternatively, the casement
35 layers 3 may be constructed from a rigid material such as

stainless steel and bolted or clamped together.  Incorporated
within at least one or both of the substrate layers there are
at least two ducts 6.   These ducts are separated by a thin
partition 90.   The same ducts join a third duct the cross-
5   sectional area of which is equal to the summed cross-sectional
area of the two ducts 6. At a minimum distance 91 from the
juncture of ducts 6 and 7 third duct 7 joins, axially, a
fourth, segmented flow duct 10 that is of cross-sectinal area
and width at least 25% that of the third duct 10, and most
10   preferably 50% of that of the third duct.   The geometrical
restriction 9 in the common duct 7 reduces the cross-sectional
dimensions so that the laminar flow streams within the common
duct convert to segmented flow 8 within the adjoining segmented
flow duct 10.  The fluid manipulation device or assembly
15   incorporates one or more radio frequency transponders 11
embedded within its matrix or attached to the external surface.
In FIGS 1B-1E one or more openings 12 within the substrate
layers may be provided and connected to the external
environment with input and/or output ducts 13 for the transfer
20   of fluids.  In FIGS 1C-1D a second fluoropolymer-based or
-coated substrate is omitted and instead a fluoropolymer
coating 14 is applied to the layer of casement material that
lies in intimate juxtaposition with the first substrate layer.
FIG 1D shows a fluid manipulation device as in FIG 1C but where
25   a thin metallic film 15 is deposited on to the non-machined
parts of the substrate layer and casement layer. This film may
be caused to rise in temperature by inductive heating and weld
the substrate layer to the casement material that lies in
intimate juxtaposition with it. FIG 1E shows a cut-away plan
30   view of the fluid manipulation device showing features common
to all variants of the fluid manipulation device, with the
exception of optional input-output fluid communication ducts
13.
        FIGS 2A-2B show a fluid manipulation device similar to
35   FIG 1 but where no separate casement material is provided. A

first fluoropolymer-based or -coated substrate 1 and a second
fluoropolymer-based or -coated substrate 2 are held in intimate
juxtaposition by means of thermocompressive welding or
inductive heating of an optional metallic film 16 deposited on
5   the non-duct surfaces, of at least one of the substrate layers,
to at least 5°C above the glass transition temperature of the
fluoropolymer film or bulk material and subsequently cooled to
ambient.

FIG 3 shows a preferred embodiment of the device for the
10  manipulation of fluids. Casement layers 3 are provided with
peripheral inter-digitated, interlocking, concentric semi-
circumferential fins 17 that enable the casement parts to join
in a specific geometrical configuration, and allow for an
enhanced mechanical binding between mating layers. Casement
15  protrusions 4 reach through vias in the substrate layers and
join corresponding protrusions from the opposed casement layer.
The casement layers may be provided with one or more
through-holes 18 which allow additional functionalities. The
through-holes may provide visual access to the fluidic ducts in
20  substrate layers 1 or 2 where the nearest substrate layer is
configured to allow optical access, for example for a charged
coupled device. The through-holes are provided with an annular
recessed undercut 19, which can be used to retain additional
substrate devices fitted with a corresponding peripheral rim.
25  The device may be provided with one or more additional
supporting layers 20 situated either side of the juxtaposed
substrate layers 1,2.

FIGS 4A-4B show another preferred embodiment of the
device for the manipulation of fluids. More than one substrate
30  layers 1 are stacked on top of each other in an assembly.
Substrate layers may have ducts machined in one side or both.
Fluidic communication between layers may be provided by
individual through-layer via holes translocating fluids from
one layer to the next or any other. Equally, fluid
35  communication to two or more layers may share a common opening

for connection with the external environment which communicates
to each layer through openings in each layer that are in turn
aligned vertically through the stack of substrate layers as
indicated in FIG 4B. Such a vertical common duct 22 may act as
5   an output drain for fluids emerging from one or more segmented
flow ducts (not shown in FIG 4 for clarity, but see 10 in FIG
1) on two or more substrate layers. The assembly may
accommodate a variable number of substrate layers by
incorporating a casement layer spacer 23 which may be thermally
10  joined or bolted to casement layers 3.

FIGS 5A-5D show another preferred embodiment of the
device for the manipulation of fluids. The Figure shows a
device similar to that described in FIG 4 but where the
through-holes 18 in casement 3 incorporate one or more
15  smaller substrate devices (called hereafter daughterboards 24
as described in more detail in FIG 6) or multilayer
daughterboards 35 of such. FIG 5B shows side and plan views of
a daughterboard showing the distal surface 36 with protrusions
27 and the proximal surface 37 with through vias 28. FIG 5C
20  shows a single layer daughterboard 24 and a multilayer
daughterboard 35. The multilayer daughterboard may be formed
from a daughterboard substrate with daughterboard interlayers
38 stacked on top of one another. The daughterboard
interlayers may be provided with a range of through vias
25  aligned with the substrate layer protrusion vias 28 for the
passage of fluids between layers or additional through vias for
further fluid communication between layers or alternate sides
of given interlayers. A further daughterboard capping layer 39
may be provided which contains no through vias but may
30  incorporate ducts for the passage of fluids. FIG 5D shows a
fluid handling device as described more fully in FIG 4 but
where the casement through holes have been occupied with an
example daughterboard 24 and a daughterboard substrate assembly
35.

35       FIG 6 shows a preferred embodiment of the daughterboard.

Each daughterboard comprises a substrate base 25 fitted with a recessed rim 26 so that it can be secured in place by the recessed undercut 19 in casement 3 through-holes 18 (see FIG 3 for 3, 18, 19). A daughterboard may comprise one or more
5  protrusions 27 from the base, in each of which protrusions a via 28 may be provided. The daughterboard substrate vias may function for the passage of fluids, or with appropriate coatings the guided passage of light through a liquid core within the via, or with appropriate metallic or conductive
10  polymer coatings the communication of electrical power or data. The daughterboard may have machined within its proximal surface one or more ducts 29 for the passage of fluids. The daughterboard substrate may be bonded to another daughterboard capping layer 30 (called herein daughterboard capping layer) in
15  which further ducts 31 may be provided for the passage of fluids. The daughterboard substrate layer may be provided with cavities 32 accessible from the distal side in which can be placed sensor and actuator components. The daughterboard substrate layer may be provided with an optional alignment
20  ridge 33 that can mate with a corresponding groove (not shown for clarity) in the daughterboard capping layer. The daughterboard protrusions 27 may be provided with compressible fluoroelastomeric sealing rings 34 to help provide hermetic seals when interfaced with the fluid manipulation device.

25       FIG 7 shows another preferred embodiment of a fluid manipulation device. In this embodiment, fluid inputs are made from centralised fluid communication ports and segmented fluid flow through the network of ducts is caused by centrifugal means. Immiscible fluids are caused to move from an external
30  source through fluid communication ducts 13 to and through the ducts 6 to a common duct 7 containing a geometrical restriction 9, or other device to cause segmentation of laminar fluid streams, and into the segmented flow duct 10. The segmented flow stream in duct 10 may be further divided into
35  two or more segmented flow ducts which exit the fluid

manipulation device directly from the substrate layer 3 through a peripheral exit port 40 located at the periphery of the device or through a duct 13 in casement 3 on either the proximal or distal surface. Two or more segmented flow ducts
5    may merge into one common duct draining segmented flow streams from the device.

FIG 8A shows two ducts 6 along which immiscible fluids are caused to move into a common duct 7 which incorporate a geometrical constriction 9 that converts laminar flow in common
10   duct 7 to segmented flow 8 in segmented flow duct 10. The relative volumes of the immiscible phase liquid components present in the segmented flow stream may be controlled by the relative flow rates of the immiscible phase liquids. FIGS 8B-8C show variants of FIG 8A but where the relative proportions of
15   the immiscible phase liquids in the segmented flow duct have been altered by the flow rates of the same liquid phase components through the input ducts 6. This enables liquids to be eluted in the segmented flow duct(s) as serial aliquots of defined volume.

20   FIG 9A shows two ducts 6 along which immiscible fluids are caused to move into a merged common duct 7 which incorporates a segment 41 provided with a change in surface energy that converts laminar flow in common duct 7 to segmented flow 8 in segmented flow duct 10. FIG 9B shows two ducts 6
25   along which immiscible fluids are caused to move into a merged common duct 7 which incorporates a segment provided with a photo-responsive surface 42 in contact with the fluid and of which surface the contact angle varies upon illumination 43 so causing the laminar flow in common duct 7 to convert to
30   segmented flow 8 in segmented flow duct 10. The effect of illumination is to cause a temporally defined and reversible photo-wetting effect. FIG 9C shows two ducts 6 along which immiscible fluids are caused to move into a merged common duct 7 which incorporates a segment provided with two electrically
35   conductive surfaces 44 separated by an insulator, which could

33

be the wall of the duct or a thin interelectrode film, with one
of the surfaces in contact with the liquids in common duct 7.
Upon electrical polarisation with an applied voltage 45, the
contact angle varies causing the laminar flow in common duct 7
5    to convert to segmented flow 8 in segmented flow duct 10. The
effect of polarisation is to cause a temporally defined and
reversible electro-wetting effect.

FIGS 10A-10B show two ducts 6 along which immiscible
fluids are caused to move into a  merged common duct 7 which
10   incorporates  a  cruciform  junction  46  or  staggered-T  or
staggered-side (shown in FIG 10B) junction with other ducts 47
that function to provide electro-kinetic disruptions of the
parallel fluid flow in common duct 7 causing the conversion to
segmented flow 8 in duct 10. Electrical potentials are applied
15   between the ducts 47 to cause electrokinetic flow across duct
7.

FIGS 11A-11B show examples of a preferred fluid duct
configuration for the fluid manipulation device where in FIG
11A two ducts 6 merge to form a common duct 7, and where in FIG
20   11B three ducts 6 merge to form a common duct 7. In both
examples laminar flow predominates within common duct 7 until
the point of geometrical constriction 9 is reached which causes
laminar flow to convert to segmented flow 8 in segmented flow
duct 10. It will be realised by those practised in the art,
25   given the benefit of this disclosure, that more than three
ducts may also be merged to form a common duct, so providing a
means to produce streams of segmented fluids where even more
than three immiscible phase components are present. In
addition, a  step-change in surface energy, photowetting,
30   electrowetting  and  electrokinetic  disruption  of  laminar
immiscible phase flow (as shown in FIGS 9A, 9B, 9C, 10A and
10B) may be employed to form segmented flow instead of
constriction 9.

FIG 12 shows a further example of another fluid duct
35   configuration of the device for the manipulation of fluids as

shown and described in FIGS 8A-8C, but with the addition of
another geometrical constriction 48 within the segmented flow
duct 10. This has the effect to cause a change in two- and
three-dimensional morphology of the fluid segments 8 as they

5   pass through the constriction. This may convert, for example,
ovoid fluid segments (as suggested in 8) to longer and thinner
segments 49. The constriction 48 may be repeated in a serial
manner, so incrementally altering the morphology of fluid
segments.

10         FIG 13 shows a further example of another fluid duct
configuration of the device for the manipulation of fluids.
Here segmented flow stream 8 is generated as in FIG 1E but,
equally, the same may be generated according to the duct
configurations shown in FIGS 9-11. The segmented flow duct

15  splits at junction 50 into two or more smaller ducts 51,
narrower in width, depth, both or diameter, causing the fluid
segments to divide into smaller segments in each of the
provided ducts 51. Optionally, the segmented flow duct 10 may
be widened, as shown at 52, prior to splitting into the two or

20  more smaller ducts, allowing for fluid segments to assume a
morphology more conducive to splitting at the junction 50.

           FIG 14 shows a further example of another fluid duct
configuration of the device for the manipulation of fluids.
Here segmented flow stream 8 is generated as in FIG 1E but,

25  equally, the same may be generated according to the duct
configurations shown in FIGS 9-13. The segmented flow duct 10
is joined by one or more tributary ducts 53 at right angles or
any other angle allowing for additional liquids to pass into
the segmented flow duct. This feature facilitates the

30  sequential exposure of fluid segments to further liquids as
they are caused to move along the segmented flow duct.

           FIG 15 shows a further example of another fluid duct
configuration of the device for the manipulation of fluids. In
FIG 15A fluid segments 8 are caused to flow in segmented flow

35  duct 10. Segmented fluids may then be caused to flow into an

expansion duct 54 where the internal dimensions are expanded such that fluid segments, previously of a non-spherical morphology (as indicated in 8), assume a spherical geometry 55 where the periphery of the spherical segments no longer makes

5   physical contact with the internal walls of the duct. By this means sequences of spherical droplets are formed.

It will be understood by those practiced in the art, given the benefit of this disclosure, that sequences of near-identical spheres can be created, or alternatively

10  sequences of custom size spheres, by altering the relative flow rates of the immiscible phase fluids caused to flow into the common duct. FIG 15B shows a similar example where the bulk material or outer layer(s) of the spheres thus created may be cross-linked or polymerised by exposure to electromagnetic

15  radiation 56, such as ultraviolet light. Fig 15C shows another similar example where the bulk material or outer layer(s) of the spheres thus created may be caused to solidify, gel, freeze, polymerise, crosslink or otherwise to assume a less than completely liquid state by exposure to additional reagents

20  fed from a tributary duct 57. Equally, the spheres may remain in a liquid form but become exposed to or enveloped by other compounds provided by the liquid added through the tributary duct. It will be recognised by those skilled in the art, given the benefit of this disclosure, that more than one tributary

25  duct may be provided in a serial succession thus enabling the opportunity to make sequential exposures to more than one additional reagent as may be required in a particular chemical reaction, or required for the provision of layered coatings around each sphere. Linear arrows denote direction of fluid

30  movement. Waved arrows denote direction of exposure of electromagnetic radiation.

FIG 16 shows a further example of another fluid duct configuration of the device for the manipulation of fluids. The segmented flow duct 10 may be provided with a tributary duct 61

35  that makes parallel contact over a short geometrical section 60

such that the fluids in both ducts flow in parallel in a shared duct for a short duration. In Figure 16A a contactor duct contains an aqueous liquid phase 59 (shaded black). At the merger of the two ducts the aqueous component 59 of the
5   segmented fluid in the segmented fluid duct 10 is attracted to the aqueous fluid 59 in the contactor duct and flows out through the continuation 62 of contactor duct with the rest of the aqueous fluid. The segmented fluid is thereby reduced to a singular stream of the other non-aqueous phase. In FIG 16B the
10  reverse is demonstrated whereby at the merger of the two ducts the non-aqueous component 58 of the segmented fluid in the segmented fluid duct is attracted to the non-aqueous fluid 58 in the contactor duct 61 and flows out with the rest of the non-aqueous fluid through continuation 62 of the contactor
15  duct. The segmented fluid is thereby reduced to a singular stream of the aqueous phase. The configuration of fluid ducts provides a means of reverting the segmented flow stream back to the original separate phase components.

FIG 17 shows a further example of another fluid duct
20  configuration of the device for the manipulation of fluids. The gas-liquid phase segmented flow in duct 10 may be interfaced with another duct 66 but separated at the juncture by a gas permeable material 63 such as a membrane. The gas phase component 65 (shaded black) of the segmented flow stream in
25  duct 10 rapidly diffuses across membrane 63 and escapes through duct 65. This causes the segmented flow in duct 10 to revert to a continuous stream of liquid phase component in continuation of fluid duct 10 beyond the interface with duct 66.

FIG 18 shows a further example of another fluid duct
30  configuration of the device for the manipulation of fluids. The device shown is configured such that two separate aqueous fluid streams 67, 68 are caused to segment with an immiscible fluid phase 72 at a constriction junction within separate common ducts 7. The two separate segmented flow streams 8 are caused
35  to move along separate segmented flow ducts 10 and which meet

at junction 69. The segmented flow streams are appropriately synchronised by means of pumping systems applied to cause the aqueous segments to meet and merge at the junction 69, to form larger aqueous segments 70 separated by the immiscible phase
5   components which also merge to form larger segments 71. The rapid internal circulation vortex flow within the fluid segments 70 and 71 cause very rapid mixing. The aqueous segments or other immiscible phase components may comprise similar solutions incorporating dissolved or suspended
10  compounds/components of similar or dissimilar concentrations. By the means of controlled volume elution and rapid micromixing achieved with the device configuration, a wide range of the dilution and/or mixing protocol may be achieved. The Figure shown illustrates the device where two segmented flow streams
15  are merged, but others with more than 2 segmented flow streams may be constructed.

FIG 19 shows a further example of another fluid duct configuration of the device for the manipulation of fluids. Ducts 6 provide immiscible fluids which merge as parallel flow
20  in common duct 7 before forming segmented flow 8 in segmented flow duct 10 after constriction 9. One of the ducts 6 is joined by another duct 73 which feeds an additional stream of the same liquid phase component. In the example shown, two fluid streams 74 and 75 of the same phase merge and form parallel flow in the
25  merged duct 76. The output from the merged duct 76 in turn merges with two more ducts 6 containing immiscible phase 72 to form a common duct 7. The constriction 9 in the common duct 7 causes the laminar flow of fluids 74, 75 and 72 to reform as segmented flow 8 in segmented flow duct 10. This geometrical
30  configuration provides a means for the precision controlled elution and mixing of similar or dissimilar fluid streams, and is readily controllable over a wide range of flow rate conditions.

FIGS 20A-20D show a further example of another fluid
35  duct configuration of the device for the manipulation of

38

fluids. The device is provided with one or more pressure sensors in the form of capillary gas-filled manometers. These are constructed as blind tributary ducts 80 arranged at any angle between 0° and 180° to the first microfluidic duct 81

5 along which fluids are caused to move. The first microfluidic duct may be any duct formed on the device for manipulation of fluids where there is non-segmented flow. The tributary ducts are preferably filled with inert gas such as argon or helium. Fluid within the first fluidic duct will rise into the

10 tributary duct according to the prevailing pressure conditions. The tributary ducts function according to the principle that a change in gas volume provides a change in fluid pressure, i.e. an increase in fluidic pressure gives a decrease in gas volume. Therefore, by knowing the cross sectional area and length of

15 the blind tributary ducts, the initial volume can be calculated and with the displacement of the fluid in the channel, the increase/ decrease of the pressure-affected volume can be calculated. With liquid in the first microfluidic duct 80, the liquid level within the tributary duct 80  rises to an initial

20 pressure setting Pi. According to pressure changes (for example, during fluid movement caused by the application of pressure) the pressure of the fluid increases and the fluid moves further up the tributary duct changing the volume of the trapped gas, thus giving P1, P2, P3 pressure readings (FIG

25 20B). Other geometrical arrangements of more than one tributary duct 80  can provide information on fluid flow rate by indicating  the pressure drop over predetermined length (FIG 20C) or over a fluid resistor caused by a constriction 82  in the first fluid duct (FIG 20D). The capillary manometers may be

30 provided in a linear, serpentined, curved or coiled format and meniscus movements visualised by an optical device such as a charged coupled device. Arrows denote direction of fluid flow.

In any of the segment-generating devices in accordance with the invention, the inlet or supply passages for the

35 respective fluids may be devoid of functionally operative input

ports and so act as closed reservoirs (although these passages
may have input ports which are used during manufacture and then
sealed).   Thus, the fluids are stored until caused to flow
along their respective passages and combine to form a segmented
flow:  the segmented flow may pass out of the device through an
output port, either to waste or to another device, or may pass
to a reservoir in the same device.